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The nonextractive immunoassay techniques developed with enzyme immunoassay procedures are being applied to fluorescent immunoassay visualization. Sorbed contaminants can be viewed using this modified fluorescent immunoassay techniques and and epifluorescent microscope to observe the microdistribution of sorbed contaminants.

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AUGMENTATION AWARD FOR MONOCLONAL ANTIBODY DETECTION OF CHLORINATED BENZENES ON CONTAMINATED SEDIMENTS

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30 September 1994

AASERT Technical Report

Prepared for
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Progress of AASERT Student

The AASERT student has been using a Zeiss Axiophot epifluorescent microscope to examine the feasibility of fluorescent immunoassay (FIA) techniques for visualization of contaminants sorbed to soil particles. The first task completed was to select a fluorochrome which would be compatible with the soil FIA. In the excitation/emission wavelength ranges used in common fluorochromes, the mineral particles exhibit significant yellow-green autofluorescence. The fluorochromes commercially available conjugated to secondary antibodies are fluorescein, rhodamine, phycoerythrin, and Texas Red™. At present, the excitation/emission filter set which is needed for Texas Red is not available in the laboratory. Fluorescein emits in the yellow-green range and rhodamine was too weak an emitter. Work completed during the first grant year used R-phycoerythrin (RPE), which emits in the orange range, for fluorescent immunoassay examination of soil contamination.

A variety of soil sample types were examined with the FIA method: brick crushed to be retained on sieve #45, brick #80, builders sand #45, kaolinite, crushed obsidian #170, St. Peter's sandstone #45, St. Peter's sandstone #80, S-2 soil (obtained from Soil Testing Laboratory at Kansas State University) #400, and S-2 soil #200. The contaminant sorbed on the soils was 2,4-dinitrobenzene sulfonate (DNB), and the primary antibody was anti-DNB developed in rabbit (rb $\bar{a}$DNB) (Sigma). The samples were contaminated by soaking the soil samples in a pH 10 DNB solution. The labelled secondary antibody was commercially-obtained antibodies to rabbit developed in goat labelled with R-phycoerythrin (gt $\bar{a}$rb-RPE). All results are photodocumented with daylight ASA400 Ektachrome slide film.

Clean and contaminated samples are first blocked using a protein solution to prevent nonspecific attachment of antibodies during the procedure. The samples are then incubated and agitated in the primary antibody solution (rb $\bar{a}$DNB). The samples are then washed to remove unbound antibodies, and the fluorochrome-labelled secondary antibody (gt $\bar{a}$rb-RPE) is added, and the sample and solution are incubated and agitated. The sample is washed again, and can then be viewed under the epifluorescence microscope.

There is a distinction between contaminated samples and uncontaminated samples when treated with FIA. The distinction was clearly observed in the brick samples (which exhibit low autofluorescence) and the St. Peter's sandstone. There was less distinct difference in the obsidian and S-2 soil, and no discernible difference between contaminated and uncontaminated samples using the kaolinite and builders sand. Experiments were conducted varying the blocking concentration (used to reduce nonspecific binding of the antibodies which will yield false positives), and two different blocking solutions were tried. Chicken egg albumin block reduced autofluorescence, but did not appear to prevent non-specific binding. Skim milk block autofluoresced somewhat, but fulfilled the primary goal of preventing nonspecific binding. Therefore skim milk has been used as the protein component of the block solution in the experiments.

An oral presentation titled "Fluorescent Immunoassay Visualization of Sorbed Pollutants" was given by W.K. Moore (AASERT student) at the Ninth Annual Hazardous Waste Research Conference, 8-10 June 1994, Montana State University, Bozeman, Montana.

Academic Performance of AASERT Student

The graduate student supported under the AASERT grant has maintained a 4.0/4.0 grade point average during the first grant year.